Developing a Fluorescence Assay to Test the Effects of Saccharin on Gram-Negative and Gram-Positive Bacteria

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Background

- More than 98% of the human gut microbiome consists of bacteria that either belong to the *Firmicutes* or *Bacteroidetes* phyla [1].

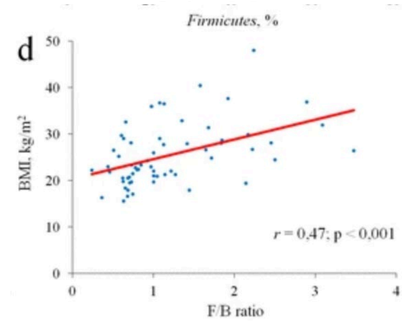


Figure 1. An individual's BMI is proportional to their F/B ratio [2].

- A gram staining protocol using SYTO 13 and HI will be implemented to quantify the viability of the bacteria.
- Fluorescent labeling provides more robust identification of bacteria than CFUs.
- **Hypothesis**: Bacterial suspensions with a larger concentration of *S. aureus* will more intensely fluoresce red than those with a larger concentration of *E. coli*.

Materials and Methods

Grow *S. aureus* & *E. coli*

↓

Create Bacterial Solutions

↓

Centrifuge

↓

Add Stains

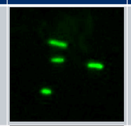
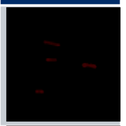
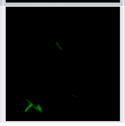
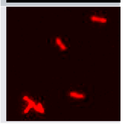
↓

Incubate

↓

Microplate Assay

Figure 2. Expected colors of fluorescence of SYTO 13 and HI.

	SYTO 13	HI
Gram-Negative Bacteria		
Gram-Positive Bacteria		

- SYTO 13 & HI diffuse into the cell and bind to DNA
- HI quenches SYTO 13 [3]

Centrifuge

- Gram-negative: *E. coli*
- Gram-positive: *S. aureus*

Results

Figure 3. Fluorescence increased in accuracy after double centrifugation.

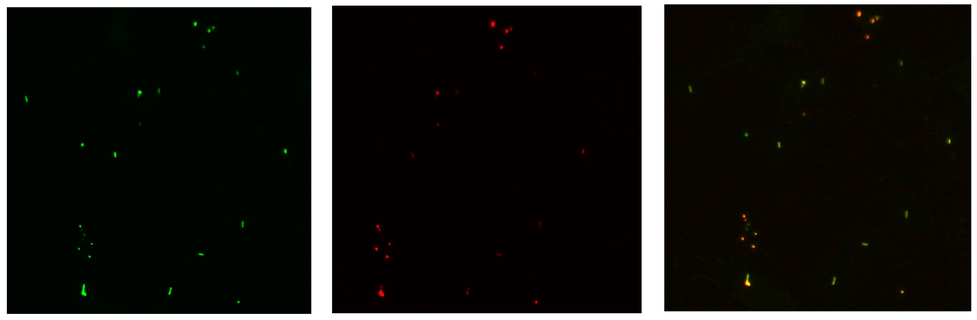


Figure 4. Tube 12 viewed under a fluorescent microscope.

Conclusions

- The accuracy of fluorescence increased after double centrifugation, not necessarily the intensity.
- This could be attributed to staining the entire bacterial suspension, not just the bacteria.
- HI quenching was best visualized when suspensions were double centrifuged.

Figure 5. Microfuge tubes before (left) and after (right) double centrifugation with stains.

- Will carry forward centrifugation before microplate assay. This provided more accurate fluorescence readings.

Future Directions

- Further address the lack of linearity in red channel
- Apply fluorescent labeling protocol to *L. casei* and *B. vulgatus*
- Independently inoculate *L. casei* and *B. vulgatus* with saccharin
- Inoculate *L. casei* and *B. vulgatus* with saccharin in a co-culture

Literature Cited

[1] Thomas, François, et al. "Environmental and gut bacteroidetes: the food connection." *Frontiers in microbiology* 2 (2011): 93.

[2] Koliada, Alexander, et al. "Association between body mass index and Firmicutes/Bacteroidetes ratio in an adult Ukrainian population." *BMC microbiology* 17.1 (2017): 120.

[3] Mason, David J., et al. "A fluorescent Gram stain for flow cytometry and epifluorescence microscopy." *Appl. Environ. Microbiol.* 64.7 (1998): 2681-2685.

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